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(71) Applicant: CASE WESTERN RESERVE UNIVERSITY [US/US]; 2040 Adelbert Road, Cleveland, OH 44106 (US).

(72) Inventors: SUKENIKK, Chaim, N. ; 3662 Bendemeer Road, Cleveland Heights, OH 44118 (US). CULP, Lloyd, A. ; 2323 Canterbury Road, University Heights, OH 44118 (US).

(74) Agents: MURTAUGH, John, P. et al.; Pearne, Gordon, McCoy & Granger, 1200 Leader Building, Cleveland, OH 44114 (US).

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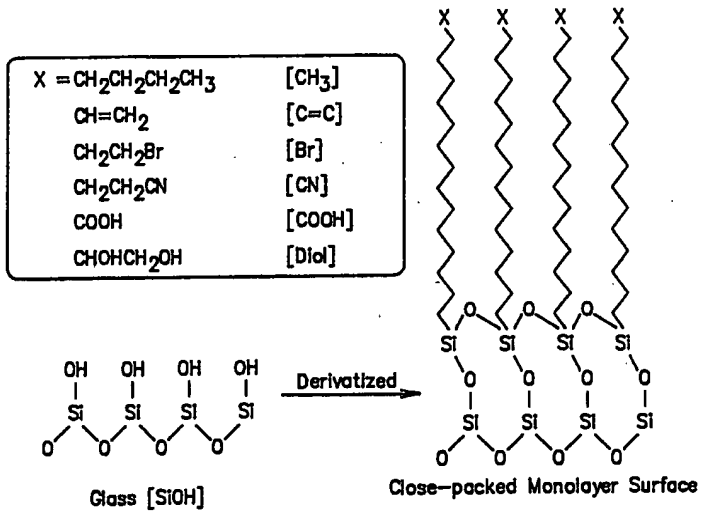

(57) Abstract

A process for selecting the types of cells that will grow on a structure, such as an implantable device or a cell growth surface. The implantable device may have a titanium surface. The process includes attaching a molecular monolayer to the surface of the structure. The monolayer has a functional group at its distal end. The possible function groups include CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$. The monolayer is coated with an adhesion-mediating molecule such as fibronectin. Cells then contact the coating. The character of the functional group affects the growth characteristics of the adhering or contacting cell, independently of the nature of the underlying structure. Also disclosed is a method of preparing a metallic surface such as titanium to receive a molecular monolayer. The surface is placed in hot water (40-50 °C) for 4 hours with sonication, or in boiling water for 8 hours without sonication.

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"Process For Controlling Cell Growth On Surfaces"

BACKGROUND OF THE INVENTION

The present invention relates in general to cell growth and, in particular, to a method for controlling cell growth on a surface utilizing a molecular monolayer.

Description of Related Art

It is difficult to prepare a surface of a substrate or structure in such a way that a particular type of cell will attach to and grow on that surface at selective advantage. It is important to develop an ability to prepare such surfaces in order to produce such things as workable and effective devices for implantation into the body. If the cells of the tissue in which the implantable device is implanted will not grow onto the surface of the implant, causing a knitting between the implantable device and the body, problems can develop with the implant and the implantation may fail. For example, an implantable device with a porous surface is described in U.S. Patent No. 3,855,638, which is incorporated herein by reference. A metallic implantable device, such as a hip implant with its surface covered with tiny projections or posts for tissue ingrowth, is described in U.S. Patent No. 4,608,052, which is also incorporated herein by reference. However, these techniques suffer from the fact that the body may recognize the metal as a foreign material and produce a fibrous layer between the body and the implant, preventing a close knit between the body and the implant.

Another effort to deal with this problem has been to coat the implant with a bone-like calcium phosphate crystal called hydroxyapatite, which the body may accept as a non-foreign bone material. However, to the extent hydroxyapatite is useful, it is useful only in connection with bone-forming cells. It cannot be tailored to enhance or repress the growth of different, specific cell types in other tissues. Moreover, good adhesion of hydroxyapatite to titanium metal can be a problem.

It is also important to be able to control the growth of particular types of cells in contact with a surface, for example, to be able to enhance the growth of one type of cell and repress or inhibit the growth of another type. For example, with regard to a device implanted into cartilage, it would be useful to enhance the growth of fibroblasts (to knit with the cartilage) and inhibit growth of neuroblasts (nerve cells, which would not be useful in that situation). In this example, the fibroblasts and neuroblasts are the preselected cell types, and the growth of each type is controlled.

SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a process for selecting the types of cells that will grow on a particular surface. As used in this specification and the claims herein, cell growth means cell survival, cell division, and/or cell differentiation. The process comprises first coating the surface with a molecular monolayer and providing a preselected functional group at the distal end

thereof. A layer of plasma fibronectin or other adhesion-mediating molecule is then coated onto the molecular monolayer. The substrate thus prepared will affect and control the growth characteristics of different cell types in contact with that surface. As a result, growth of certain types of cells which would facilitate tissue ingrowth and knitting between implant and body can be enhanced, and growth of other types of cells on the surface can be repressed or inhibited. A cell growth surface is a surface upon which cell growth may take place, and includes a glass slide, a petri dish, a 24-well dish, and an industrial bioreactor with beads, baffles and/or stirrers therein. For example, in a laboratory, cell culture may be grown on surfaces in a Corning Pyrex Slow Speed Stirring Vessel, #26501-1L, containing therein Kontes Cytocarriers. Implantable devices include devices implantable in humans as well as devices implantable in animals. As used in this specification and the claims herein, adhering includes both active and passive attachment.

The present invention finds utility (a) in the field of body implants and prosthetics, particularly implantable devices made of titanium, (b) in applications involving bio-repulsive surfaces for implants and moving parts of prosthetics, as well as more controlled bio-adhesive surfaces for the structure of the prosthetic device, and also (c) in the field of cell and tissue growth, where containers and laboratory dishes and glassware with preselected surface characteristics can control, enhance, repress, and otherwise mediate growth of preselected cell types and cultures. Surface treatments that enhance the rate of cell attachment and growth would be a major benefit to both research laboratories and to the scaled-up production of specific cell lines and cell-derived materials. Many aspects of the foregoing discussion and invention are disclosed in Lewandowska, K., Balachander, N., Sukenik, C. N., and Culp, L.A.; "Modulation

of Fibronectin Adhesive Functions for Fibroblasts and Neural Cells by Chemically Derivatized Substrate," Journal of Cellular Physiology, 141:334-345 (1989), the contents of which are hereby incorporated by reference herein in their entirety.

Titanium is used increasingly as an implant material. Among other reasons, its mechanical properties are closer to those of bone than are stainless steel and cobalt-chromium alloys. Coatings or surface alterations that promote cell attachment and regulate physiological response would make titanium even more useful. Implants made of metals other than titanium could also be coated using reasoning and procedures similar to those described herein to control cell attachment and regulate cell-type specific physiological response.

Thin organic, molecular monolayer films offer an excellent method for the modification of surface properties. A high level of molecular monolayer uniformity can usually be achieved using a carbon chain at least 14 carbons long excluding the functional end group. However, somewhat shorter carbon chains may be successful in this application. Carbon chains containing 22 carbon atoms have been successfully prepared in other applications, and it is believed that carbon chains of similar length, or longer, may be used herein. The carbon chain is typically polymethylene to assure sufficient chain flexibility for assembly and packing. However, polymethylene chains in this application can tolerate, and are meant to include, the incorporation of double bonds, an aromatic ring, a limited number of hetero-atoms, and/or halogenated substituents or segments.

Self-assembly of $SiCl_3$-terminated long-chain amphiphiles forms well-ordered, siloxy-anchored, crosslinked monolayers, as described in U.S. Patent No. 4,539,061 to Sagiv, the contents of which are hereby incorporated by

reference herein. However, Sagiv does not teach any method of consistently preparing a titanium surface so that it will accept a molecular monolayer. A clean titanium surface, unprepared in accordance with the present invention, will generally not accept or bond to a molecular monolayer as described in Sagiv. The present disclosure teaches a solution to this problem, which comprises increasing the number of hydroxy groups available for reaction on the metallic surface by maintaining the metallic surface in contact with boiling water for a sufficient period of time, or with water at a temperature of more than 40 degrees Centigrade with sonication for a sufficient period of time.

The modification of titanium surfaces with covalently-attached, self-assembled monolayers offers many advantages. Since the coating process involves dipping the surface being treated into a dilute, homogeneous solution of surfactant in an organic solvent, it is versatile and can be applied to materials and implants of almost any configuration. Coating of already fabricated implants and prostheses would thus be readily achieved. Since the monolayer film so completely isolates the substratum from the outside environment, it also permits the creation of surfaces with specific properties on various bulk materials. Finally, the ease with which such surfaces can be transformed by conventional organic chemistry allows the creation of surfaces with the functionality needed to impart desirable chemical and physical properties. The stability, uniformity, and manipulability of these surfaces should all combine to make them useful in the design of new biomaterials.

It is also believed that any oxide or hydroxide-bearing surface similar to glass or titanium may be expected to undergo chemistry and biochemistry similar to that described herein. Thus, we believe that molecular monolayers may be applied to a wide range of surfaces.

Various functional groups have been incorporated into the surface of these very uniform molecular monolayer assemblies. See Balachander, N., and Sukenik, C.N., "Functionalized Siloxy-Anchored Monolayers With Exposed Amino, Azido, or Cyano Groups," Tetrahedron Letters, 29:5593-5594 (1988), the contents of which are incorporated by reference herein. A surface to which is attached a molecular monolayer having a preselected functional group at its distal end is referred to as a "derivatized surface."

The ability of these monolayers to effectively isolate their substrate is clear. Since not all functional groups can coexist with the $SiCl_3$ group needed to anchor the monolayer, surfactants or monolayer precursor molecules containing a chemically modifiable group that can coexist with the $SiCl_3$ group have been developed. Given these materials and the stability of the siloxy-bound monolayer, in situ generation of yet additional functionality can be achieved.

Adhesion-mediating molecules include several proteins that have cell-type-specific receptors for selected cell populations. Fibronectin, as an extracellular matrix glycoprotein, is an adhesion-mediating molecule that mediates adhesion of many mesenchymal and some non-mesenchymal cells to their collagen environment. This occurs by the binding to fibronectin of (a) glycoprotein receptor complexes on the cell surface called "integrins," as well as of (b) heparan sulfate proteoglycans on the cell surface. This facilitates the complete physiological response from some cells. Laminin is also an adhesion-mediating molecule.

Study of the molecular mechanisms by which fibronectins bind to artificial matrices and whether the composition of surface biomaterials can modulate the biological activities of fibronectins coated thereon has been limited,

in contrast to more extensive studies of the binding of other blood-borne proteins, particularly fibrinogen.

In accordance with the present invention, it has been shown that the binding of plasma fibronectin to derivatized glass and titanium surfaces alters fibronectin conformation such that cell growth of animal (including human) cells such as mesenchymally-derived fibroblasts and nervous system-derived neuroblastoma cells adhering to the fibronectin is modulated in distinctive ways. These altered responses are cell-type-specific; that is, fibroblast changes on fibronectin-coated surfaces were different from those of neuroblastoma cells. Mouse Balb/c 3T3 cells are an excellent model of fibroblasts that come from many tissues of both human and non-human animal species. In addition, Platt neuroblastoma cells are an excellent model for the differentiation processes of some neuron populations that come from human and non-human species. The adhesion-mediating processes of mouse Balb/c 3T3 cells are essentially identical to those of normal (non-malignant) fibroblast cells. The adhesion-mediating processes of Platt neuroblastoma cells are essentially identical to those of normal (non-malignant) neuron-derived cells. Accordingly, it is believed that the results of these studies are applicable to normal (non-malignant) fibroblast and neuron-derived cells that occur in the body of human and non-human animal species.

It has also been shown that it makes no difference whether the underlying surface is glass or titanium; as long as the derivatized monolayer is the same, the cell response will be the same. Thus, the underlying substratum cannot "act at a distance" to affect receptor-dependent responses from cells. Chemical end groups that directly interface bound fibronectin molecules clearly dominate cell responses. Thus, it is believed that when different materials have their

surface derivatized in the same way, the response from any particular type of cell will be the same.

These and other aspects of this invention are more fully described in the following specification.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 illustrates a molecular model of derivatized substrate;

FIG. 2 illustrates the binding of plasma fibronectin to substrata;

FIG. 3 illustrates the quantitation of cell attachment on substrata;

FIG. 4 illustrates the quantitation of neurites on substrata;

FIG. 5 illustrates a molecular model of monolayer functionalized surfaces on glass and on titanium; and

FIG. 6 illustrates the relative degree of adsorption of plasma fibronectin to glass and titanium surfaces.

DESCRIPTION OF THE PREFERRED EMBODIMENT

EXAMPLE I - Responses Are Cell-Type-Specific On Derivatized Glass Surfaces.

1. Summary

Plasma fibronectin was adsorbed onto glass surfaces derivatized with an alkyl chain and six chemical end groups interfacing with the bound plasma fibronectin. The response of fibroblasts (Balb/c 3T3 cells) and human neuron-derived (Platt neuroblastoma) cells adhering to the plasma fibronectin was examined. Using new derivatization protocols, the following surfaces were tested in order of increasing polarity: [CH_3], [C=C], [Br], [CN], [Diol], [COOH], and underivatized glass [SiOH]. For all substrata, plasma fibronectin bound equivalently, using either a supersaturating amount of plasma fibronectin or a subsaturating amount in competition with bovine albumin. Attachment of both cell types was also equivalent on all substrata. However, spreading/differentiation responses varied considerably.

Spreading and differentiation are characteristics of cell growth and development. The reorganization and formation of F-actin stress fibers in 3T3 cells is correlated with cell growth.

While stress fibers formed effectively on plasma-fibronectin-coated [SiOH] and [Br] substrata, only small linear bundles of F-actin and a few thin stress fibers were observed on the [COOH], [Diol], and [CN] substrata: the hydrophobic substrata ([CH_3] and [C=C]) gave an intermediate response. When a synthetic peptide containing the Arg-Gly-Asp-Ser sequence required for integrin binding to fibronectins was included in the medium as an inhibitor, additional differences were noted: Stress fiber formation was completely inhibited on [SiOH] but not on [Br] and stress fiber formation was very sensitive to inhibition on the

hydrophobic substrata, while the F-actin patterns on the [CN] and [COOH] substrata were unaffected.

Neurite outgrowth by neuroblastoma cells is characteristic of the specialized differentiation functions of neuronal cells. Evaluation of neurite outgrowth by neuroblastoma cells on these substrata revealed both qualitative and quantitative differences, as follows: [Diol] = [COOH] > [SiOH] > > [CN] = [Br] > [CH3] = [C=C]. While there was poor cytoplasmic spreading and virtually no neurites formed on the hydrophobic surfaces when plasma fibronectin alone was adsorbed, neurite formation could be "rescued" if a mixture of plasma fibronectin with an excess of bovine albumin was adsorbed, demonstrating complex conformational interactions between substratum-bound plasma fibronectin and adhesion-inert neighboring molecules.

In summary, these experiments demonstrate that different chemical end groups on the substratum modulate, control, enhance, repress, and/or inhibit functions for cell adhesion, growth, and their specialized differentiation functions, principally by affecting the conformation of these molecules rather than the amounts bound. Furthermore, these experiments confirm multiple-receptor interactions with the fibronectin molecules in cell-type-specific adhesion patterns.

2. Materials and Methods

a. Cells and growth conditions

Balb/c 3T3 (clone A31) cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% neonatal calf serum, penicillin, and streptomycin in 10% CO_2: humidified air (Lewandowska et al., J. Cell Biol., 105:1443-1454 (1987). Human neuroblastoma cells (Platt), grown under the same medium and conditions, are constitutive for production of small neurites characteristic of neural tumor cells

(Vallen et al., J. Cell. Physiol., 135: 200-212 (1988). Human neuroblastoma neurites are particularly useful in these experiments and are easy to enumerate because of their long linear neurite extension over surfaces. They require a complex array of signals from the fibronectin in order to achieve growth cone migration on the substratum.

For experiments, cells were detached at confluence, after rinsing twice with phosphate-buffered saline (PBS), using 0.5 mM EGTA in PBS at 37°C for 30 minutes (Lewandowska et al., J. Cell Biol., 105:1443-1454 (1987)). After rinsing twice, cells were resuspended in DMEM plus 250 ug/ml heat-treated bovine serum albumin (BSA; referred to as "adhesion medium").

b. Derivatization of Surfaces

Glass coverslips were derivatized via surface Si-OH linkages, as previously described (Netzer and Sagiv, J. Am. Chem. Soc., 105:674-676 (1983); Balachander and Sukenik, Tetrahedron Lett., 29:5593-5594 (1988)), and as illustrated in FIG. 1. FIG. 1 illustrates glass coverslips derivatized by the attachment of a functionalized 14-carbon aliphatic chain to the surface-available silicon atoms. Briefly, a siloxane network covalently anchors an array of hydrocarbon chains terminating with one of the end groups [X] interfacing the medium. [X] is the active component in the binding reactions of fibronectin. [CH_3] was obtained using octadecyltrichlorosilane and [C=C], [Br], and [CN] resulting from $SiCl_3$-terminated compounds derived from 16-bromo-1-hexadecene. Deposition of the self-assembled monolayer films was achieved by dipping glass coverslips, cleaned by an Argon plasma, into 20 mM solutions of the $SiCl_3$ derivative in dicyclohexyl for 2-5 minutes, achieving maximal derivatization as ascertained below. [COOH] and [Diol] were obtained

by $KMnO_4$ oxidation of [C=C] either with or without added base, respectively.

Characterization of surfaces was based on 1) wettability (contact angle measurements), 2) Fourier transform infrared spectroscopy (samples prepared on silicon prisms) in the attenuated total reflectance mode (FTIR-ATR), and 3) electron spectroscopy for chemical analyses (ESCA). [CH_3] and [C=C] surfaces are both hydrophobic and oleophobic (advancing water contact angles > 110° for [CH_3] and > 105° for [C=C]; hexadecane contact angles of 40-42°). FTIR-ATR spectra show the expected polymethylene chains and (for [CH_3]) the terminal CH_3 group (2,960 cm^1). [CN] and [Br] have water contact angles of 74° and 81°, respectively, and show the expected ESCA signals for the heteroatom ([CN] N at 403 eV; [Br] Br at 72 eV, uncorrected for shift caused by insulator substrate). [CN] on silicon ATR prisms have an infrared absorption at 2,247 cm^{-1}. The [Diol] and [COOH] surfaces, derived from the hydrophobic [C=C] monolayers, were hydrophilic (water contact angles of 30° and 52°, respectively).

c. Fibronectin and its adsorption

Human plasma fibronectin was purified from plasma by affinity chromatography (Lewandowska et al., J. Cell Biol. 105:1443-1454 (1987) and stored in CAPS buffer at -80°C. For adsorption to surfaces, plasma fibronectin was diluted to 20 ug/ml in PBS, and 500 ul of this solution was added to each well of 24-well cluster dishes containing derivatized glass coverslips for 1 hour at 37°C (Haas and Culp, J. Cell. Physiol., 113:289-297 (1982); Haas et al., J. Cell Physiol. 120:117-125 (1984)). For coverage of coverslips, medium containing heat-treated BSA was added for 1 hour at 37°C. Saturability of fibronectin binding was tested using a goat polyclonal antiserum to human plasma fibronectin and an

enzyme-linked immunosorption assay (ELISA) as previously described (Lewandowska et al., FEBS Lett. 237:35-39 (1988)). BSA was adsorbed similarly and tested by ELISA as well, using a polyclonal antiserum to bovine albumin.

d. Adhesion Assays

EGTA-detached 3T3 (10×10^4) or Platt (5×10^4) cells were inoculated into wells containing adhesion medium and plasma fibronectin-coated, derivatized glass coverslips. To quantitate attachment, cells had been previously radiolabeled by incorporation of [^{3}H] thymidine into DNA (Lewandowska et al., J. Cell Biol. 105:1443-1454 (1987); Mugnai et al., J. Cell Biol., 106:931-943 (1988). After 1 hour, unattached cells were rinsed out, attached cells rinsed twice with PBS, and attached cells solubilized with a NaOH/SDS mixture for quantitation of radioactivity in a scintillation counter. Standard errors of multiple determinations were calculated.

To evaluate morphological responses, cells were allowed to attach and spread for 4 hours (3T3 cells) or for 16 hours (Platt cells) for optimal neurite development. They were then fixed with 3% glutaraldehyde for photography on a Nikon Diaphot microscope using Kodak technical pan 2415 film. Neurites generated with Platt cells were quantitated as described previously (Waite et al., Exp. Cell Res., 169:311-327 (1987); Mugnai et al., J. Cell Biol., 106:931-943 (1988)). For higher resolution and evaluation of neurites, scanning electron microscopy was also performed (Mugnai et al., J. Cell Biol. 106:931-943 (1988); Mugnai et al., Eur. J. Cell Biol., 46:352-361 (1988)).

To evaluate microfilament networks, 3T3 cells spreading for 4 hours were fixed with 3.7% formaldehyde in PBS for 20 minutes and then treated as previously described (Laterra et al., J. Cell Biol., 96:112-123 (1983)) to bind

rhodamine-phalloidin to the F-actin-containing networks. Stained coverslips were inverted into 50% glycerol:PBS and evaluated in the Nikon Diaphot microscope with fluorescence illumination (photographed under an x100 objective with Kodak 2475 recording film).

3. Results

a. Fibronectin Binding to Surfaces

Plasma fibronectin binding to derivatized substrate (FIG. 1) was tested by ELISA. FIG. 2 illustrates plasma fibronectin (pFN) binding to substrata. Wells of 24-well tissue culture cluster dishes, containing glass coverslips with the indicated derivatization of the coverslip, were adsorbed for 1 hour at 37°C with either 20 ug/ml human plasma fibronectin (stippled bars) or a mixture of 2.5 ug/ml plasma fibronectin plus 17.5 ug/ml bovine serum albumin (BSA); slashed bars. Wells were rinsed with PBS and coverslips were transferred to another 24-well cluster dish and postadsorbed with 250 ug/ml heat-treated BSA for 1 hour at 37°C. After rinsing the wells with PBS, the amount of plasma fibronectin bound was assayed by ELISA, using goat polyclonal antihuman plasma fibronectin, as described by Lewandowska et al., FEBS Lett. 237:35-39 (1988). The final ELISA reactions stopped with 5 M NaOH after 1 hour were transferred to 96-well dishes for assaying absorbance at 405 nm in an ELISA reader. Standard errors of multiple determinations are shown.

As shown in FIG. 2, using an excess of plasma fibronectin (20 ug/ml) for saturating substrata (Hughes et al., Exp. Cell. Res., 121:307-314 (1979); Haas and Culp, J. Cell. Physiol., 113:289-297 (1982); Lewandowska et al., FEBS Lett. 237:35-39 (1988), plasma fibronectin bound comparably to all surfaces as evaluated by the Student's t test. Binding was also examined when a limiting amount of plasma fibronectin was competing with a large excess of albumin (a

molecule that cells do not interact with). When plasma fibronectin (2.5 ug/ml) was mixed with an excess of bovine albumin (17.5 ug/ml) prior to adsorption of the mixture to surfaces (FIG. 2), plasma fibronectin still bound effectively and competitively achieved a concentration on the substratum similar to that of plasma fibronectin alone, indicating the effectiveness of plasma fibronectin interaction with all surface end groups in competition with albumin. Two exceptions were noted with the [CH_3] and [COOH] substrata with a smaller amount of plasma fibronectin bound. (However, this small reduction could not be an explanation for altered cell responses as shown below).

b. Cell Attachment To Plasma Fibronectin-Coated Surfaces

Attachment of thymidine-radiolabeled cells was determined. When bovine albumin was adsorbed, both 3T3 and Platt cells failed to attach at all, demonstrating the inability of cells to interact with this protein on all derivatized substrata and requiring an adhesion-promoting protein to mediate physiologically compatible cell responses.

With regard to FIG. 3, Balb/c 3T3 fibroblasts and Platt neuroblastoma cells were radiolabeled separately in stock cultures by incorporation of [^3H] thymidine into their DNA in complete medium as described above in Materials and Methods. After chasing the radiolabeled precursor for 24 hours, cells were detached from stock culture, washed by repeated resuspension/centrifugation, and enumerated: 10 x 10^4 3T3 cells or 5 x 10^4 Platt cells were inoculated into 24-well dishes containing derivatized glass coverslips coated with 20 ug/ml plasma fibronectin and adhesion medium. After 1 hour to permit maximal attachment, unattached cells were rinsed out and the adherent cells solubilized in NaOH/SDS as described by Mugnai et al., J. Cell Biol., 106:931-943 (1988), for determination of radioactivity by scintillation

counting. An equivalent sized aliquot of the cell suspension was also assayed for radioactivity to determine the percentage of radiolabeled cells adherent. In FIG. 3, standard errors are shown for multiple determinations.

As shown in FIG. 3A, when attachment had plateaued, 3T3 cells attached equivalently to all derivatized substrata coated with plasma fibronectin. The same was true for neuroblastoma cells (FIG. 3B). This contrasted with the ability of both 3T3 and Platt cells to attach to all substrata in the absence of any adsorbed plasma fibronectin or any other protein (data not shown); however, cells adherent to naked surfaces failed to respond further and detached within 6-12 hours in all cases, demonstrating the physiological incompatibility of all surfaces without an appropriate adhesion-promoting protein (Grinnell, Int. Rev. Cytol. 53:65-144 (1978)).

c. Spreading and Cytoskeletal Responses of 3T3 Cells

In contrast to the equivalency of attachment, cytoplasmic spreading and differentiation of cells were significantly different among substrata. Reorganization of microfilaments (F-actin) into stress fibers by fibroblasts on fibronectin requires complex reactions, including transmembrane signaling from fibronectin to both heparan sulfate proteoglycans (Laterra et al., J. Cell Biol. 96:112-123 (1983) and the glycoprotein integrin (Tamkun et al., Cell, 46:271-282 (1986) on the cell surface. Burridge et al., Annu. Rev. Cell Biol. 4:487-525 (1988). Therefore, stress fibers and focal contacts on the substratum are a diagnostic indicator of the complete response of fibroblasts permitting subsequent movement, cell division, and expression of genes linked to anchorage dependence (Dike and Farmer, Proc. Natl. Acad. Sci. U.S.A., 85:6792-6796 (1988)).

With regard to F-actin cytoskeletal reorganization in adherent 3T3 cells, 3T3 cells were detached from stock cultures and washed by repeated resuspension/centrifugation. Cells (7.5×10^4) were inoculated into 24-well dishes containing derivatized glass coverslips coated with 20 ug/ml plasma fibronectin and adhesion medium; they were permitted to spread and to reorganize cytoskeletal networks for 4 hours as described previously (Laterra et al., J. Cell Biol., 96:112-123 (1983); Hall et al., Exp. Cell Res., 179:115-136 (1988)). The adhesion medium and unattached cells were rinsed out of wells that were rinsed three times with PBS prior to paraformaldehyde fixation and staining of cells with rhodamine-phalloidin as described in Materials and Methods. Coverslips were photographed under epifluorescence illumination for the same exposure times on a Nikon Diaphot microscope using Kodak 2475 recording film. All negatives were also processed identically to allow direct visual comparisons among the samples; in some cases (e.g., samples [SiOH] and [Br]), this resulted in overexposed images in order to visualize samples with much poorer organization (e.g., samples [COOH] and [CN]. F-actin stress fibers formed extensively in cells on plasma fibronectin-coated glass. Thinner and shorter F-actin bundles were principally observed on the plasma fibronectin-coated carboxy substratum, while a small subset of cells therein appeared to contain some thin stress fibers. On the cyano substratum coated with plasma fibronectin, very short F-actin bundles could be observed in poorly spread cells, with some modest thin stress fibers evident at the periphery of better-spread cells. On the hydrophobic substratum represented by the methyl end group coated with plasma fibronectin, thicker stress fibers were observed in many cells and some cells had formed extensive stress fibers. The plasma fibronectin-coated bromo substratum gave cells with extensive stress fiber arrays virtually

identical to the control glass surface, while the olefin hydrophobic surface gave some cells with excellent stress fibers.

As previously mentioned, Balb/c 3T3 cells were examined at a 4-hour time point when cytoplasmic spreading had optimized. Rhodamine-phalloidin stains extensive stress fibers formed on plasma fibronectin-coated [SiOH]. In contrast, cells on highly polar [COOH] and [Diol] surfaces had greatly reduced F-actin organization with linear bundles of limited distance and lacking the extensive pattern shown with respect to [SiOH]. A similar pattern was observed on [CN] surfaces. Some thin fibers could be identified in approximately one-third of the cells on both [COOH] and [CN] substrata. Hydrophobic substrata represented by [CH_3] yielded a stress fiber pattern similar to the [SiOH] control in one subpopulation of cells and some thinner fibers evident in a second subpopulation of cells. The [Br] response was virtually indistinguishable from [SiOH] with thick stress fibers evident throughout. The [C=C] response was very similar to the [CH_3] response, demonstrating consistency for the two hydrophobic surfaces. These analyses indicate that transmembrane signaling processes from fibronectin on these surfaces are significantly different. The most polar surfaces represented by the [COOH], [Diol], and [CN] gave the poorest F-actin responses; the hydrophobic surfaces of [CH_3] and [C=C] an intermediate response; and the [Br] surface a pattern virtually indistinguishable from the control [SiOH]. The most reasonable explanation, considering the comparable amounts of plasma fibronectin bound to all surfaces, is differing conformations of the plasma fibronectin leading to differing interactions with multiple cell surface receptors (see below as well). These patterns remained unchanged at time points up to 24 hours, demonstrating the stability of cytoskeletal reorganization.

F-actin reorganization was also examined with a complementary approach involving use of an inhibitor. The discussion with respect thereto is contained in Lewandowska et al., Journal of Cellular Physiology, 141:334-345 at 337-340, 342-44 (1989), the contents of which are incorporated herein by reference in their entirety. The results of the inhibitor study confirmed the differing binding relationships of plasma fibronectin on derivatized substrata with the integrin complex as the cell surface. Since cells were treated uniformly in this paradigm, these results support the belief that plasma fibronectin on these substrata has differing conformations with varying interactions with cell surface receptors (such as integrins and heparan sulfate proteoglycans).

d. Spreading and Neurite Outgrowth of Neural Cells

Cells derived from the neural crest of the embryo can extend neurites on fibronectin in many cases (Rovasio et al., J. Cell Biol., 96:462-472 (1983)). There are several binding domains in fibronectin that may regulate this neuritogenesis (Mugnai et al., J. Cell Biol., 106:931-943 (1988)). Therefore, neuritogenesis was tested on derivatized substrata to determine whether fibronectin conformational changes generate all-or-none or intermediate responses from such cells.

With regard to neuritogenesis of Platt neuroblastoma cells on substrata, Platt human neuroblastoma cells were detached from stock cultures by EGTA treatment, as described in Materials and Methods. After washing the cells, 5×10^4 cells were inoculated into 24-well dishes containing derivatized glass coverslips coated with 20 ug/ml plasma fibronectin and adhesion medium. Cells were allowed to develop neurite processes over an 18-hour period. Adherent cells were fixed with glutaraldehyde and photographed under phase

contrast microscopy using Kodak film on a Nikon Diaphot microscope. Thin extending neurites were very evident in the cells on plasma fibronectin-coated glass. Neurites were even more evident in a higher percentage of cells on the plasma fibronectin-coated diol substratum. In contrast, cells on the plasma fibronectin-coated cyano substratum spread poorly and, for the most part, failed to extend neurites. On the plasma fibronectin-coated methyl substratum, thicker processes were extending over the substratum from some cells; and this was also the case with the olefin surface. On the plasma fibronectin-coated bromo substratum, a small percentage of cells were extending along thin neurites, while the majority of the cells were not extending processes.

Thus, human Platt neuroblastoma cells responded to plasma fibronectin-coated [SiOH] in an overnight incubation by spreading in a bipolar fashion; a sizable percentage of cells extended neurites. On [Diol] and [COOH] substrata, these cells displayed identical patterns, with neurites evident in many calls. In contrast, cells on [CN] had greater spreading with few neurites. The hydrophobic surface, [CH_3], gave an intermediate response with more effective spreading, more bipolarity, and some thicker but shorter neurites. [Br] yielded a response identical to [CN], while [C=C] was virtually identical to [CH_3]. These results confirm that conformational differences of plasma fibronectin bound to these substrata lead to very different spreading and differentiation patterns of cells adhering thereto. Furthermore, neural cell responses were different from those reported above for 3T3, demonstrating that neural cells and fibroblasts rely on different binding activities of plasma fibronectin on substrata to achieve their respective phenotypes.

Neurites were then quantitated (Mugnai et al., J. Cell Biol., 106:931-943 (1988)). Non-neural 3T3 cells give

a "background" level of process extension in this assay of 4-5%. With regard to quantitation of neurites or substrata as illustrated in FIG. 4, Platt neuroblastoma cells were treated as described above with regard to neuritogenesis of Platt neuroblastoma cells on substrata. Neurite-bearing cells were enumerated as defined by Mugnai et al, J. Cell Biol., 106:931-943 (1988) and their percentage in the total adherent cell population determined. The background level of neurite-bearing cells in a non-neural cell population, such as Balb/c 3T3 cells evaluated under the same conditions, was routinely 4-5% (Id.). The standard errors of multiple determinations are shown.

For Platt, there are marked differences in the percentage of neurite-bearing cells on these substrata, as seen in FIG. 4. They are comparably low for the two hydrophobic, the bromo, and the cyano substrata, with no statistical differences among them. [SiOH] yielded twice as many neurite-bearing cells above the 3T3 background as the hydrophobic ones, [COOH] three times, and [Diol] four times the percentage. Therefore, the most polar substrata gave the ideal conformation of plasma fibronectin for maximal differentiation of cells via the binding of one or more cell surface receptors.

e. Albumin "Rescue" of Fibronectin Functions on Hydrophobic Surfaces

Since hydrophobic substrata gave the poorest neurite responses from Platt cells, an experiment was designed in which plasma fibronectin would be present on the substratum along with a second neutral protein, i.e., a protein that had effective hydrophobic interactions such as serum albumin. Therefore, [C=C] derivatized coverslips were coated with either 20 ug/ml plasma fibronectin, or with a mixture of 2.5 ug/ml plasma fibronectin and 17.5 ug/ml BSA

(see Materials and Methods), in which plasma fibronectin binding had been tested earlier by ELISA (see FIG. 2). Platt neuroblastoma cells (5×10^4) were inoculated into all wells containing adhesion medium and incubated 18 hours to permit neurite extension. The plasma fibronectin-only coating yielded poor spreading for most cells; only a rare cell formed neurites. In contrast, on plasma fibronectin, BSA substrata cells responded more effectively by spreading and becoming bipolar; many cells now generated neurites. The same results were obtained for [CH_3]. Therefore, the conformational alterations of plasma fibronectins alone on the hydrophobic substrata can be "reversed" by neighboring interactions with "adhesion-neutral" and hydrophobic proteins bound to the same surface. Conformation of plasma fibronectins is determined not only by interactions with the chemical end groups on inert substrata but also by interactions with neighboring proteins bound to the same surface.

4. Discussion

These results provide evidence that fibronectin functions can be modulated by chemical end groups of the inert substratum to which fibronectin is bound. Substrata of all six chemical groups adsorbed the same amounts of fibronectin when compared with underivatized glass, including a case in which plasma fibronectin competes with an excess of albumin for binding (i.e., an 8:1 mass excess and a 28:1 molar excess). Since a diverse series of end groups were used, the binding of fibronectin must occur through a multiplicity of amino acid side-chain interactions with substrata, including hydrogen bonding, van der Waals interaction, and ionic interactions, any one of which may be sufficient for binding. Furthermore, these studies demonstrate that fibronectin binding and saturation levels are independent of the

polarity of substrata end groups and that fibronectin function for the two cell types is altered in different ways, again not stringently linked to polarity. Although attachment of cells was observed on substrata in the absence of any adsorbed protein, cells failed to spread, detachment occurred, and cell death was noted in all cases, demonstrating the necessity for an adhesion-promoting protein such as plasma fibronectin to facilitate physiologically compatible responses from cells.

With either fibroblasts or neural cells, attachment levels were equivalent on all six derivatized substrata when compared with underivatized glass. Cell surfaces harbor several classes of molecules that can mediate binding to fibronectin substrata to facilitate attachment processes only (including the glycoprotein integrin class, the heparan sulfate proteoglycans, and the highly sialylated gangliosides). These data indicate that at least one of the binding domains along substratum-bound fibronectin molecules is available for interactions with one or more of these surface molecules in all cases.

However, cytoplasmic spreading and differentiation require transmembrane signaling from surface receptors that bind coordinately to fibronectin and to cytosolic elements within the cell. In both the 3T3 and neuroblastoma systems, chemically derivatized substrata modulate the functions of fibronectins by altering their conformation, and thereby their interactions, with the panel of cell surface "receptors." This evidence can be summarized as follows (Table 1).

TABLE 1

Summary of Cell Responses to Different Plasma Fibronectin-Coated Derivatized Surfaces

Surface Group	Stress Fibers[1]	Neurites[2]
Hydrophilic		
[Diol]	+	+ + +
[SiOH]	+ + +	+ +
[COOH]	+	+ + +
[CN]	+	+
[Br]	+ + +	+
Hydrophobic		
[C=C]	+ +	+
[CH_3]	+ +	+

[1] + Only thin fibers observed;
+ + Both thin and thick fibers present;
+ + + Only thick fibers observed.

[2] + + + High neurite counts;
+ + Moderate neurite counts;
+ Low neurite counts.

First, reorganization of F-actin into stress fibers in 3T3 cells varies significantly among the seven substrata (Table 1). [SiOH] and [Br] substrata provided optimal stress fiber formation throughout the cytoplasm of all cells. The hydrophobic surfaces ([CH_3] and [C=C]) gave an intermediate response with both thick and some very thin stress fibers. The polar surfaces ([CN], [COOH], and [Diol]) gave the poorest response, with numerous star-shaped clusters of F-actin in the cytoplasm, some short linear bundles of F-actin, and, in a few cases, some very thin stress fibers.

Second, the pattern of neurite formation in neuroblastoma cells was quite different from the patterns of stress fiber formation in 3T3 cells (Table 1). The quantitative and qualitative evaluations of neurites can be placed into the following array: [Diol] = [COOH] > [SiOH] > > [CN] = [Br] > [CH_3] = [C=C]. Therefore, cell types of very different embryological origin, i.e., fibroblasts as represented by the model 3T3 cell and neuronal cells represented by the derivative neuroblastoma cell, reacted with substrata in cell type-specific ways. F-actin reorganization in the 3T3 cells was reasonably effective on the hydrophobic substrata, while these substrata were the poorest for neurite formation of neuroblastoma cells. The [Br] substratum yielded an excellent stress fiber pattern in 3T3 cells, but was poor for eliciting neurites from the neuroblastoma cells.

These data indicate that the conformation of fibronectin molecules can be highly variable on chemically derivatized substrata, and that subsequent interactions with multiple cell surface receptors are affected. Cell surface receptors probably interact synergistically with multiple binding domains on the intact plasma fibronectin. Since only intact plasma fibronectin was tested in these studies and considerable differences in F-actin reorganization were documented, it appears that the topology of binding domains in fibronectins is critical for maximal cell surface response during adhesion.

Of significance as well was the demonstration of the "rescue" of defective fibronectin function by heterologous neighboring proteins on the substratum. In the present experiments, hydrophobic substrata generated fibronectin conformations, when this was the only protein bound, extremely ineffective for neurite formation by neural cells. However, albumin molecules along with plasma fibronectin on these substrata reverted the conformation of plasma fibronectin

such that receptor interaction (therefore, neurite extension) was maximal. This system will be useful for resolving the fibronectin-dependent binding mechanisms critical in neurite differentiation in various neuronal populations and for identifying the cell receptors involved.

The dimeric fibronectin molecule exhibits complex binding properties as it interacts with inert substrata containing various end groups. These end groups can modulate the functions of fibronectins during their reaction with a multiplicity of cell surface receptors. This level of regulation and control of adhesion-promoting proteins and the cells adhering thereto is important with regard to the effectiveness of biomaterial interactions with differing biological systems, such as implants in a body. In this regard, differing cell and tissue types from the body or animal are predicted to respond differently, based on the parameters observed in this study.

EXAMPLE II - Responses Are Cell-Type-Specific on Derivatized Titanium Surfaces In The Same Way As On Derivatized Glass Surfaces

1. Summary

The surface of titanium has been modified by covalent attachment of an organic molecular monolayer anchored by a siloxane network. The titanium surface often requires enhancement prior to attachment of the monolayer. This monolayer coating completely covers the metal and allows controlled modification of surface properties by modification of the exposed chemical end groups of the monolayer-forming surfactant.

When glass and titanium are derivatized with the same chemical end groups and coated with plasma fibronectin, and preselected cell types are adhered thereto, the responses are cell-type-specific, as discussed above, and are independent of the character of the substrate as glass or titanium. Identical surfaces are obtained on the glass and titanium; only the monolayer coating interacts with the environment. Surfaces bearing each of four different chemical end groups were used; see FIG. 5. The $[CH_3]$-, [Br]-, and $[CH=CH_2$-terminated monolayers were directly formed from surfactants containing those groups and the [Diol] surface was obtained by oxidation of the $[CH=CH_2]$ monolayer. The surface was completely derivatized with a stable, close-packed monolayer with the indicated structure.

2. Materials and Methods

a. Solvents and Reagents

Dicyclohexyl (Aldrich) was vacuum-distilled and passed through Activity I alumina (3% water by weight). Doubly distilled water was used. Hexadecane was passed through Al_2O_3 to remove polar contaminants. Octadecyl trichlorosilane (Aldrich) was vacuum-distilled before use to

deposit the [CH_3] surfaces. w-Undecenyl alcohol (Aldrich) was converted into w-hexadecenyl bromide (Balachander et al., Tetrahedon Lett., 29:5593-5594 (1988)). This sixteen-carbon chain with an olefin at one end and a CH_2Br unit at the other is used to make w-hexadecenyl-trichlorosilane (for [C=C] surfaces, Netzer et al., J. Am. Chem. Soc., 105:674-676 (1983)) or 1-bromo-16-trichlorosilyl hexadecane (for [Br] surfaces, Balachander et al., Tetrahedron Lett., 29:5593-5594 (1988)).

b. Preparation of Solid Substrates

Both square (22 x 22 mm) and round (to fit 24-well cluster dishes) glass slides were used. They were cleaned by washing with doubly distilled water, followed by Soxhletting hot $CHCl_3$ for 1 hour. Titanium (Ti 540, 0.004 gauge from Teledyne Rodney Metals, CA) was sonicated in hot water (40-50°C) for 4 hours, washed in acetone, and Soxhletted in hot chloroform for 1 hour.

If titanium is not treated with a technique such as the hot water to enhance the oxide coating, some samples will not accept or bond to a molecular monolayer as described in Sagiv. Heating the titanium in water at 40-50°C for 4 hours with sonication, or heating it in boiling water without sonication for 8 hours, provided successful monolayer attachment for all titanium samples tested. This procedure builds up the oxide layer and hydrates the surface of the titanium, resulting in an adequate concentration of Ti-OH moieties on the surface; see FIG. 5.

The substrates were dried in an oven, cleaned for 30 minutes in an r.f. Argon plasma (Harrick PDC-3xG Plasma Cleaner), and stored in fluorocarbon containers (Fluoroware) and used within 1 to 2 days.

c. Monolayer Coating Solutions

All trichlorosilane surfactants were used as 0.02-0.025 M solutions in dicyclohexyl. The surfactant was added to dicyclohexyl under inert atmosphere and transferred to the bench top. All surfactant solutions were used within 1 to 3 hours after their preparation. Monolayers were prepared by holding the substrate (glass or titanium) with Teflon-coated tweezers and immersing it into a 10 mL beaker containing the surfactant solution and a magnetic stirrer. The substrate is quickly withdrawn after 2 to 15 minutes, washed twice with $CHCl_3$ and water, and Soxhletted with hot $CHCl_3$ or 1:1 v/v $CHCl_3$/EtOH for 15 minutes.

d. Formation of Diol Surface by Oxidation of [C=C] Surface With Neutral $KMnO_4$

In a beaker was placed about 100 mg of $KMnO_4$ and 20 mL of 10% aqueous acetone. The beaker was placed in an ice bath at $0^{\circ}C$ and CO_2 bubbled through it continuously. The substrate with the [C=C] monolayer was dipped into the beaker and kept in the solution at $0^{\circ}C$ for 45 minutes. The monolayer substrate was removed, dipped in a 20% solution of sodium bisulfite in H_2O for about 15 seconds, washed with water, dried, and Soxhletted in 1:1 $CHCl_3$:EtOH for 15 minutes. These plates were characterized by contact angle measurements and showed none of the pH dependence reported for the acid surface formed by $KMnO_4$ cleavage of [C=C] (Maoz and Sagiv, Thin Solid Films, 132:135-151 (1985)).

e. Contact Angle Measurements

Contact angles were determined in a Rame-Hart Model 100 contact angle goniometer. Advancing contact angles were determined by placing a drop of H_2O or hexadecane and advancing the periphery of the drop by adding more liquid by the syringe and withdrawing the syringe and measuring the contact angle within 30 seconds. The receding contact angles were measured by first withdrawing part of the liquid from the drop. Measurements were done at ambient temperature and reported values are the average of 4 to 6 measurements taken at different points on the surface.

f. X-Ray Photoelectron Spectroscopy

XPS measurements were carried out on a PHI-Unicam Perkin Elmer instrument. Analyses were done using Mg Kα lines at a pressure of 10^{-9} torr with a take-off angle of 45 degrees. Survey spectra were recoded on a 1 mm spot, with 150 eV pass energy, 200 W electron beam power, and an acquisition time of 7 minutes. Multiplex spectra of the individual elements were carried out on a 1 mm spot, with 50 eV pass energy and a 30-minute acquisition time. Peak positions are referenced to the C 1s peak at 285 eV.

g. Animal Cell Adhesion and Growth Conditions

Human Platt neuroblastoma cells were grown in stock culture in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% newborn calf serum and antibiotics. These cells make neurites constitutively in serum-containing or protein-free media on plasma fibronectin-adsorbed tissue culture substrata. Dulbecco's medium supplemented only with 250 ug/mL heat-treated bovine albumin, referred to as "adhesion medium," is used for all animal cell adhesion experiments. Cells were treated as described in Example I. Briefly, stock cultures were rinsed free of medium and the cells

detached with 0.5 mM EGTA in phosphate-buffered saline (PBS) with gentle shaking for 30 minutes at 37°C. After three rinses with adhesion medium by centrifugation/resuspension, the final cell pellet was suspended into adhesion medium at the required concentration.

h. Animal Cell Adhesion Assays

Derivatized glass or titanium coverslips were rinsed overnight with PBS (three times) prior to their placement into 24-well tissue culture cluster dishes. Wells were adsorbed for 60 minutes at 37°C with 20 ug/mL human plasma fibronectin (purified as described in Example I) or with adhesion medium to evaluate adherence to bovine albumin-coated surfaces. (In all cases, attachment of neuroblastoma cells was minimal on albumin coatings.) The binding of plasma fibronectin to surfaces was evaluated by ELISA assay, as described in Example I, using a goat polyclonal antiserum directed to human plasma fibronectin, an alkaline phosphatase-conjugated indirect antibody, and absorbance at 405 nm. After a 60 minute adsorption of the wells with fibronectin, they were rinsed three times with PBS and postadsorbed for 60 minutes with adhesion medium to guarantee coverage of all surface sites with the non-adhesive albumin molecule. Platt neuroblastoma cells (5×10^5) were inoculated into wells and incubated for 18 hours when neurite elongation over fibronectin-coated substrata had become maximal. (In all cases, longer incubation failed to improve neuritogenesis.) Wells were rinsed three times with PBS and adherent cells fixed prior to evaluation by microscopy. Quantitation of Platt cell attachment on derivatized glass or titanium, using radiolabeled cells, as described in Example I, revealed standard errors varying from $\pm$3.5% to $\pm$5.5% for multiple determinations.

Phase contrast microscopy required 5% glutaraldehyde fixation (in PBS) of cells and examination of glass coverslips under a Nikon Diaphot microscope using Kodak 2415 film. Cells fixed on titanium were examined and photographed under epi-illumination in a Zeiss photo-microscope, using the same film. For scanning electron microscopy (SEM), coverslips were treated as described in Example I. Briefly, they were fixed in a 2% paraformaldehyde/2% glutaraldehyde mixture in 2X DMEM, dehydrated with increasing concentrations of absolute ethanol-water, critical point-dried in liquid CO_2, and sputter-coated with gold-palladium (Technics Hummer V). Coverslips were examined on a JEOL 840 SEM (tilt angle 35 degrees) and photographed with Polaroid 55 positive-negative film.

3. Results

a. Surface Properties

The monolayer coatings were initially characterized by contact angle measurements and by X-ray photoelectron spectroscopy (XPS). The contact angles for all the surfaces used in this work are given in Table 2.

TABLE 2

WATER CONTACT ANGLES (SESSILE DROP METHOD)

	Water Contact Angles	
Monolayer/Substratum	Advancing	Receding
$[CH_3]$/Glass	112	107 - 108
$[CH_3]$/Ti	110 - 112	100 - 102
[C=C]/Glass	105	98 - 100
[C=C]/Ti	103 - 106	93 - 96
[Br]/Glass	82	77 - 78
[Br]/Ti	81 - 83	65 - 70
[Diol]/Glass	30 - 34	< 10
[Diol]/Ti	26 - 35	< 10
/Bare Glass	30 - 35	10 - 15
/Bare Ti	40 - 45	10 - 15

The difference between advancing and receding contact angles (hysteresis) for a given surface is related, among other things, to the heterogeneity of the surface. The $[CH_3]$ and $[CH=CH_2]$ surfaces (FIG. 5) have advancing water contact angles of 110° and 105°, respectively, and are both hydrophobic and oleophobic. One or the other of these surfaces served as the hydrophobic test surface in each of the experiments below. The diol-terminated monolayer and the bare glass and titanium are all hydrophilic. The [Br] surface has an advancing contact angle of 82 degrees for both substrates, and is of intermediate hydrophobicity. The somewhat greater spread in contact angle values and the greater hysteresis for the titanium surfaces reflects greater surface heterogeneity and is consistent with the difference in texture and surface roughness seen in the scanning elec-

tron microscopy (SEM) of these surfaces in the animal cell adhesion study (vide infra).

XPS was used to verify the elemental composition of the monolayer. Their hydrocarbon packing was determined using the integrated intensities of the C 1s peak. Since glass is insulating, the peak positions were adjusted by fixing the C 1s peak at 285 eV. All surfaces showed the expected carbon peak and the [Br] surfaces showed the expected peak at 70.2 eV. The integrated peak intensities were consistent with comparable monolayer packing on both glass and titanium substrata and comparable packing density among the various monolayers.

b. Animal Cell Adhesion

In order to evaluate human Platt cell adhesion responses to titanium surfaces, it was important to establish the degree of plasma fibronectin binding to both underivatized and derivatized surfaces. This was done using an ELISA assay. With respect to FIG. 6, glass or titanium coverslips, either underivatized (Non deriv.) or derivatized as indicated, were adsorbed with plasma fibronectin at a concentration of 20 ug/mL or with 250 ug/mL bovine albumin on underivatized surfaces (BSA) as defined in Materials and Methods. After 1 hour of adsorption, coverslips in wells were rinsed with PBS and adsorbed fibronectin tested in an ELISA assay as described in Materials and Methods. Standard errors of multiple determinations are shown with the error bars.

As shown in FIG. 6, where a super-saturating amount of plasma fibronectin (20 ug/mL) was incubated with either glass or titanium coverslips for 1 hour, comparable amounts of fibronectin bound to both underivatized glass or titanium surfaces, as well as to the three classes of derivatized glass or titanium surfaces. As expected, albumin adsorption blocked the substratum from plasma fibronectin binding and

provided a negative control substratum to evaluate cell responses. These results indicate that alteration in cell responses cannot be ascribed to reduced levels of plasma fibronectin binding to these artificial surfaces, since fibronectin can be diluted on the substratum with bovine albumin to less than 5% of the concentrations displayed here and still yield a maximal adhesion response (Hughes et al., Exp. Cell Res., 121:307-314 (1979); Haas and Culp, J. Cell. Physiol., 113:289-297 (1982)).

Platt neuroblastoma cells were then inoculated onto various glass or titanium coverslips adsorbed with plasma fibronectin and incubated 18 hours to allow stabilization of adhesion responses and maximal neurite outgrowth. Since these neuronal cells require several different receptors to interact with different binding domains of fibronectin, this cell system is particularly sensitive to conformational changes that may occur upon fibronectin binding to various derivatized substrata. Cell attachment on all surfaces was comparable, but spreading and neurite responses mediated by transmembrane signaling processes were quite different. On underivatized glass and titanium, neuroblastoma cells became bipolar; some cells were extending, short, neurite-like processes, while some cells were extending, long, linear, thin neurites. On [Diol] surfaces of either glass or titanium, the responses were different from the underivatized controls, but similar to each other, i.e., a higher percentage of cells were extending, long, linear, thin neurites, indicating that growth cone migration over the substratum was facilitated on this particular surface whether it was on glass or titanium. Similarly, responses on the [Br] surfaces of either glass or titanium were reduced. Cell spreading was not as extensive, processes were shorter, and long, thin neurites were not observed. This was even more dramatic on the [C=C] surfaces

on both glass and titanium, where cells spread in a pseudopodial fashion, much like that of fibroblasts, and not of neural cells; and, in addition, neurite processes in bipolar cells were rarely evident. These results indicate that derivatization of glass and titanium yields similar differences in neural cell response on these plasma fibronectin-coated surfaces. Modulation of fibronectin adhesion responses is end-group-specific and independent of the underlying glass or titanium. When these surfaces were tested for cell responses in the absence of any adsorbed protein, cells attached to all surfaces for several hours without any cytoplasmic spreading, became metabolically unbalanced, and detached as dead cells. This indicates the significance of adsorption of an adhesion-promoting protein, such as plasma fibronectin, for physiologically-compatible cell responses.

These morphological changes in neuroblastoma cell responses were further documented in the scanning electron microscope (SEM). With regard to the scanning electron microscope, neuroblastoma cells were inoculated onto underivatized, [Diol]-derivatized, or [C=C]-derivatized titanium coverslips as described in Materials and Methods. The etched surface of titanium coverslips was readily apparent in all cases, and was independent of the derivatization process being used. A notable feature in the SEM images of the titanium surfaces was their etched appearance, whether they were derivatized or not, and whether they were adsorbed with plasma fibronectin or not. This indicates ultrastructural differences in the metallic surface in contrast to the smooth appearance of all glass surfaces in Example I. With respect to underivatized titanium coated with plasma fibronectin, the most common neural cell response was a bipolar cell extending two thickened neurite-like processes at both ends, and with migrating growth cones at the ends of these "neurites" containing actively ruffling plasma membrane. In contrast,

on the [Diol] titanium surface where responses were excellent, long and thin neurites were readily evident in two highly bipolar cells, and these neurites could extend >100 um from the cell bodies of cells. These excellent neurites were observed in a smaller proportion of cells on the underivatized surfaces, rarely on the [Br] surface, and virtually not at all on the [C=C] surfaces. On this last substratum, cell spreading was evident in a very different pattern: broad pseudopodial processes, reminiscent of fibroblast responses to fibronectin and not neural cells, were common for most cells. These analyses document the cellular ultrastructural changes that occur in response to similar amounts of fibronectin on various derivatized surfaces, and undoubtedly reflect the differing natures of multiple cell surface receptors interacting with substratum-bound fibronectin molecules. These differences also occur independently of the etched nature of all titanium surfaces analyzed to date, since derivatization of very smooth glass surfaces yields the same chemical end-group-specific changes in cell responses.

4. Discussion

First, plasma fibronectin binds comparably to derivatized glass or titanium surfaces; fibronectin binding is not limiting cell response. However, this binding was only tested in homogeneous solutions of plasma fibronectin.

Second, these results with animal cell adhesion responses verify that the chemical end groups facing the medium, and therefore interacting directly with fibronectin molecules bound to the surface, alter the conformation of fibronectin molecules in ways that lead to differing cell surface receptor responses from cells. Therefore derivatization of biomaterials can be used to manipulate the short term (and possibly long term) responses from select animal cell types.

Finally, there were no detectable differences between the titanium series or the glass series, revealing that the underlying substratum cannot "act at a distance" to affect receptor-dependent responses from cells. Chemical end groups that directly interface bound fibronectin molecules clearly dominate cell responses. These results support the utilization of many different biomaterials derivatized with similar approaches in order to achieve the same responses from cells.

It should be understood that various modifications, changes, and replacements of the components and methods herein may be resorted to by those skilled in the art without departing from the scope of the invention as disclosed and claimed herein.

WHAT IS CLAIMED IS:

1. A process for selecting the types of cells that will grow on a structure, and for controlling the growth thereof, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, the distal ends of said molecules being provided with a functional group;

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types; and

(c) contacting said coating of said adhesion-mediating molecule with cells and tending to selectively control the growth thereof.

2. A process according to claim 1, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

3. A process according to claim 1, wherein said adhesion-mediating molecule is fibronectin.

4. A process according to claim 1, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

5. A process in accordance with claim 1, including selecting Br as said functional group and thereby tending to enhance the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

6. A process in accordance with claim 1, including selecting one of COOH and $CHOHCH_2OH$ as said functional group and thereby tending to inhibit or repress the growth of fibroblasts and tending to enhance the growth of neuron-derived cells.

7. A process in accordance with claim 1, including selecting CN as said functional group and thereby tending to inhibit or repress the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

8. A process in accordance with claim 1, including selecting one of $CH{=}CH_2$ and CH_3 as said functional group and thereby tending to enhance the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

9. A process according to claim 1, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure and said structure includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

10. A process according to claim 1, wherein step (a) includes providing on the structure a molecular monolayer of molecules having a first functional group on the distal ends thereof, and chemically reacting said first functional group to form a second functional group.

11. A process according to claim 1, wherein said structure includes a titanium surface portion for attachment of said molecular monolayer and the step of providing said molecular monolayer on said surface portion includes pre-treating said titanium surface by contacting it with boiling water for a period of time sufficient to increase the concentration of Ti-OH moieties to enable attachment of the molecular monolayer.

12. A process according to claim 1, wherein said structure includes a titanium surface portion for attachment of said molecular monolayer and the step of providing said molecular monolayer on said surface portion includes pre-treating said titanium surface by contacting it with water at a temperature of more than $40^{o}C$, with sonication, for a period of time sufficient to increase the concentration of Ti-OH moieties to enable attachment of the molecular monolayer.

13. A process according to claim 9, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, said adhesion-mediating molecule is fibronectin, and the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

14. A method for controlling and modulating the function of an adhesion-mediating molecule associated with a structure in connection with the growth of cells contacting the adhesion-mediating molecule, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of the adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types contacting the coating.

15. A method according to claim 14, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

16. A method according to claim 14, wherein said adhesion-mediating molecule is fibronectin.

17. A method according to claim 14, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

18. A method according to claim 14, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure and said structure includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

19. A method according to claim 14, wherein step (a) includes providing on the structure a molecular monolayer of molecules having a first functional group on the distal ends thereof, and chemically reacting said first functional group to form a second functional group.

20. A method according to claim 18, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin.

21. A method for isolating a layer of an adhesion-mediating molecule from the effects of an underlying structure, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, substantially all molecules of said monolayer having a carbon chain at least fourteen carbons long; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said monolayer.

22. A method according to claim 21, wherein the molecules have a functional group at their distal ends, the adhesion-mediating molecules of said coating interacting with said functional group to provide a surface which tends to control the growth of preselected cell types contacting said coating of the adhesion-mediating molecule substantially independent of said underlying structure.

23. A method according to claim 21, wherein the adhesion-mediating molecule is fibronectin.

24. A method according to claim 21, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

25. A method for isolating a cell from the effects of an underlying structure, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, substantially all molecules of said monolayer having a carbon chain at least fourteen carbons long;

(b) causing a coating of an adhesion-mediating molecule to be formed on said monolayer; and

(c) contacting the cell with said coating.

26. A method according to claim 25, wherein step (c) includes adhering the cell to said coating for controlled cell growth in isolation from the effects of the underlying structure.

27. A method according to claim 25, wherein the molecules have a functional group at their distal ends, the adhesion-mediating molecules of said coating interacting with said functional group to provide a surface which tends to control the growth of the cell contacting said coating substantially independent of said underlying structure.

28. A method according to claim 25, wherein the adhesion-mediating molecule is fibronectin.

29. A method according to claim 25, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

30. A method for making an implantable device including a structure portion for control of cell growth, comprising the steps of:

(a) providing on the structure portion a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure portion, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types contacting the coating.

31. A method according to claim 30, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

32. A method according to claim 30, wherein said adhesion-mediating molecule is fibronectin.

33. A method according to claim 30, wherein the structure portion is comprised at least in part of a material selected from the group consisting of glass and titanium.

34. A method according to claim 30, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure portion and said structure portion includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

35. A method according to claim 34, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin, and the structure portion is comprised at least in part of a material selected from the group consisting of glass and titanium.

36. A method for making a cell growth surface, the growth surface including a support surface, the method comprising the steps of:

(a) providing on the support surface a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the support surface, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide the growth surface which tends to control the growth of preselected cell types contacting the coating.

37. A method according to claim 36, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

38. A method according to claim 36, wherein said adhesion-mediating molecule is fibronectin.

39. A method according to claim 36, wherein the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

40. A method according to claim 36, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said support surface and said support surface includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

41. A method according to claim 40, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin, and the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

42. An implantable device including a growth surface having improved ability to knit itself to surrounding tissue in a living organism, comprising:

(a) a structural portion having a support surface;

(b) a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to said support surface, said molecules of said monolayer having a functional group at the distal ends thereof; and

(c) a layer of an adhesion-mediating molecule coating said monolayer, said functional group interacting with the adhesion-mediating molecules of said layer to provide the growth surface which tends to control the growth of preselected cell types contacting said layer.

43. An implantable device according to claim 42, wherein the functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

44. An implantable device according to claim 42, wherein the adhesion-mediating molecule is fibronectin.

45. An implantable device according to claim 42, wherein the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

46. An implantable device according to claim 42, wherein said proximal ends of said molecules are attached to said support surface by means of binding siloxane groups.

47. An implantable device according to claim 46, wherein the functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and the adhesion-mediating molecule is fibronectin, and the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

48. A cell growth surface comprising:

(a) a support surface;

(b) a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to said support surface, said molecules of said monolayer having a functional group at the distal ends

thereof; and

(c) a layer of an adhesion-mediating molecule coating said monolayer, said functional group interacting with the adhesion-mediating molecules of said layer to provide the growth surface which tends to control the growth of preselected cell types contacting said layer.

49. A cell growth surface according to claim 48, wherein the functional group is selected from the group consisting of CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

50. A cell growth surface according to claim 48, wherein the adhesion-mediating molecule is fibronectin.

51. A cell growth surface according to claim 48, wherein the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

52. A cell growth surface according to claim 48, wherein said proximal ends of said molecules are attached to said support surface by means of binding siloxane groups.

53. A cell growth surface according to claim 52, wherein the functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and the adhesion-mediating molecule is fibronectin, and the support surface is comprised at least in part of a material selected from the group consisting of glass and titanium.

54. A process for the preparation of a metallic surface of an article so as to permit formation of a molecular monolayer on said surface, comprising contacting said metallic surface with water at an elevated temperature for a period of time sufficient to increase the concentration of OH moieties to enable attachment of the molecular monolayer.

55. A process according to claim 54, wherein the metallic surface is titanium.

56. A process according to claim 54, wherein said elevated temperature is approximately $100^{\circ}C$.

57. A process according to claim 56, wherein the metallic surface is titanium.

58. A process according to claim 54, wherein said elevated temperature is at least $40^{\circ}C$, and further including sonicating the article.

59. A process according to claim 58, wherein the metallic surface is titanium.

AMENDED CLAIMS
[received by the International Bureau on 09 December 1991 (09.12.91);
original claims 1-59 replaced by amended claims 1-59
(13 pages)

1. A process for selecting the types of cells that will grow on a substrate, and for controlling the growth thereof, comprising the steps of:

(a) providing on the substrate a metallic surface structure and a molecular monolayer of molecules thereon having proximal and distal ends, the proximal ends of said molecules being attached to the structure, the distal ends of said molecules being provided with a functional group;

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types; and

(c) contacting said coating of said adhesion-mediating molecule with cells and tending to selectively control the growth thereof.

2. A process according to claim 1, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

3. A process according to claim 1, wherein said adhesion-mediating molecule is fibronectin.

4. A process according to claim 1, wherein the metallic surface structure is titanium.

5. A process in accordance with claim 1, including selecting Br as said functional group and thereby tending to enhance the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

6. A process in accordance with claim 1, including selecting one of COOH and $CHOHCH_2OH$ as said functional group and thereby tending to inhibit or repress the growth of fibroblasts and tending to enhance the growth of neuron-derived cells.

7. A process in accordance with claim 1, including selecting CN as said functional group and thereby tending to inhibit or repress the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

8. A process in accordance with claim 1, including selecting one of $CH=CH_2$ and CH_3 as said functional group and thereby tending to enhance the growth of fibroblasts and tending to inhibit or repress the growth of neuron-derived cells.

9. A process according to claim 1, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure and said structure includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

10. A process according to claim 1, wherein step (a) includes providing on the structure a molecular monolayer of molecules having a first functional group on the distal ends thereof, and chemically reacting said first functional group to form a second functional group.

11. A process according to claim 1, wherein said structure includes a titanium surface portion for attachment of said molecular monolayer and the step of providing said molecular monolayer on said surface portion includes pre-treating said titanium surface by contacting it with boiling water for a period of time sufficient to increase the concentration of Ti-OH moieties to enable attachment of the molecular monolayer.

12. A process according to claim 1, wherein said structure includes a titanium surface portion for attachment of said molecular monolayer and the step of providing said molecular monolayer on said surface portion includes pre-treating said titanium surface by contacting it with water at a temperature of more than 40°C, with sonication, for a period of time sufficient to increase the concentration of Ti-OH moieties to enable attachment of the molecular monolayer.

13. A process according to claim 9, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin.

14. A method for controlling and modulating the function of an adhesion-mediating molecule associated with a substrate in connection with the growth of cells contacting the adhesion-mediating molecule, comprising the steps of:

(a) providing on the substrate a metallic surface structure and a molecular monolayer of molecules thereon having proximal and distal ends, the proximal ends of said molecules being attached to the structure, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of the adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types contacting the coating.

15. A method according to claim 14, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

16. A method according to claim 14, wherein said adhesion-mediating molecule is fibronectin.

17. A method according to claim 14, wherein the structure is titanium.

18. A method according to claim 14, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure and said structure includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

19. A method according to claim 14, wherein step (a) includes providing on the structure a molecular monolayer of molecules having a first functional group on the distal ends thereof, and chemically reacting said first functional group to form a second functional group.

20. A method according to claim 18, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin.

21. A method for isolating a layer of an adhesion-mediating molecule from the effects of an underlying structure, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, substantially all molecules of said monolayer having a carbon chain at least fourteen carbons long; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said monolayer.

22. A method according to claim 21, wherein the molecules have a functional group at their distal ends, the adhesion-mediating molecules of said coating interacting with said functional group to provide a surface which tends to control the growth of preselected cell types contacting said coating of the adhesion-mediating molecule substantially independent of said underlying structure.

23. A method according to claim 21, wherein the adhesion-mediating molecule is fibronectin.

24. A method according to claim 21, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

25. A method for isolating a cell from the effects of an underlying structure, comprising the steps of:

(a) providing on the structure a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure, substantially all molecules of said monolayer having a carbon chain at least fourteen carbons long;

(b) causing a coating of an adhesion-mediating molecule to be formed on said monolayer; and

(c) contacting the cell with said coating.

26. A method according to claim 25, wherein step (c) includes adhering the cell to said coating for controlled cell growth in isolation from the effects of the underlying structure.

27. A method according to claim 25, wherein the molecules have a functional group at their distal ends, the adhesion-mediating molecules of said coating interacting with said functional group to provide a surface which tends to control the growth of the cell contacting said coating substantially independent of said underlying structure.

28. A method according to claim 25, wherein the adhesion-mediating molecule is fibronectin.

29. A method according to claim 25, wherein the structure is comprised at least in part of a material selected from the group consisting of glass and titanium.

30. A method for making an implantable device including a structure portion for control of cell growth, comprising the steps of:

(a) providing on the structure portion a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to the structure portion, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide a surface which tends to control the growth of preselected cell types contacting the coating.

31. A method according to claim 30, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

32. A method according to claim 30, wherein said adhesion-mediating molecule is fibronectin.

33. A method according to claim 30, wherein the structure portion is titanium.

34. A method according to claim 30, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said structure portion and said structure portion includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

35. A method according to claim 34, wherein said functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin, and the structure portion is titanium.

36. A method for making a cell growth surface, the growth surface including a substrate, the method comprising the steps of:

(a) providing on the substrate a metallic support surface and a molecular monolayer of molecules thereon having proximal and distal ends, the proximal ends of said molecules being attached to the support surface, the distal ends of said molecules being provided with a functional group; and

(b) causing a coating of an adhesion-mediating molecule to be formed on said molecular monolayer for interaction with said functional group so as to provide the growth surface which tends to control the growth of preselected cell types contacting the coating.

37. A method according to claim 36, wherein said functional group is selected from the group consisting of CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

38. A method according to claim 36, wherein said adhesion-mediating molecule is fibronectin.

39. A method according to claim 36, wherein the metallic support surface is titanium.

40. A method according to claim 36, wherein said proximal ends of said molecules comprise $SiCl_3$ groups for attachment to said support surface and said support surface includes OH groups for reaction therewith and the step of providing said molecular monolayer includes reacting said $SiCl_3$ groups with said OH groups to form a binding siloxane group.

41. A method according to claim 40, wherein said functional group is selected from the group consisting of CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and said adhesion-mediating molecule is fibronectin.

42. An implantable device including a growth surface having improved ability to knit itself to surrounding tissue in a living organism, comprising:

(a) a structural portion having a support surface;

(b) a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to said support surface, said molecules of said monolayer having a functional group at the distal ends thereof; and

(c) a layer of an adhesion-mediating molecule coating said monolayer, said functional group interacting with the adhesion-mediating molecules of said layer to provide the growth surface which tends to control the growth of preselected cell types contacting said layer.

43. An implantable device according to claim 42, wherein the functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

44. An implantable device according to claim 42, wherein the adhesion-mediating molecule is fibronectin.

45. An implantable device according to claim 42, wherein the support surface is titanium.

46. An implantable device according to claim 42, wherein said proximal ends of said molecules are attached to said support surface by means of binding siloxane groups.

47. An implantable device according to claim 46, wherein the functional group is selected from the group consisting of CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and the adhesion-mediating molecule is fibronectin.

48. A cell growth surface comprising:

(a) a metallic support surface;

(b) a molecular monolayer of molecules having proximal and distal ends, the proximal ends of said molecules being attached to said support surface, said molecules of said monolayer having a functional group at the distal ends thereof; and

(c) a layer of an adhesion-mediating molecule coating said monolayer, said functional group interacting with the adhesion-mediating molecules of said layer to provide the growth surface which tends to control the growth of preselected cell types contacting said layer.

49. A cell growth surface according to claim 48, wherein the functional group is selected from the group consisting of CH_3, $CH=CH_2$, Br, CN, COOH, and $CHOHCH_2OH$.

50. A cell growth surface according to claim 48, wherein the adhesion-mediating molecule is fibronectin.

51. A cell growth surface according to claim 48, wherein the support surface is titanium.

52. A cell growth surface according to claim 48, wherein said proximal ends of said molecules are attached to said support surface by means of binding siloxane groups.

53. A cell growth surface according to claim 52, wherein the functional group is selected from the group consisting of CH_3, $CH{=}CH_2$, Br, CN, COOH, and $CHOHCH_2OH$, and the adhesion-mediating molecule is fibronectin.

54. A process for the preparation of a metallic surface of an article so as to permit formation of a molecular monolayer on said surface, comprising contacting said metallic surface with water at an elevated temperature for a period of time sufficient to increase the concentration of OH moieties to enable attachment of the molecular monolayer.

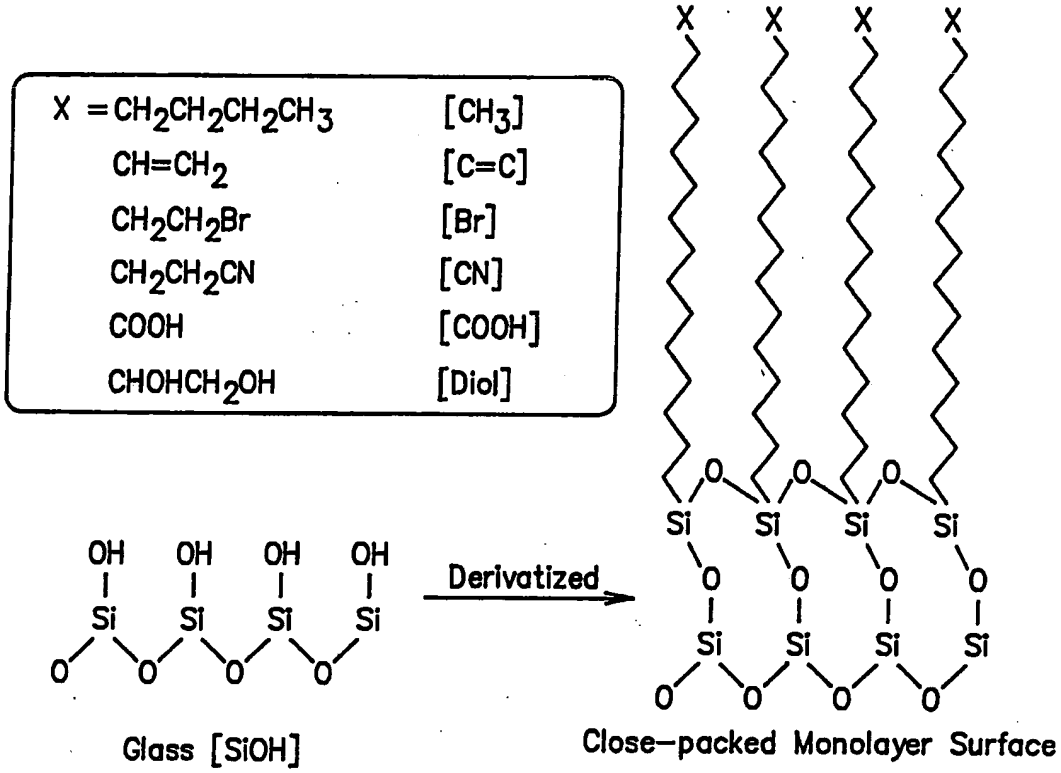
55. A process according to claim 54, wherein the metallic surface is titanium.

56. A process according to claim 54, wherein said elevated temperature is approximately 100°C.

57. A process according to claim 56, wherein the metallic surface is titanium.

58. A process according to claim 54, wherein said elevated temperature is at least 40°C, and further including sonicating the article.

59. A process according to claim 58, wherein the metallic surface is titanium.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CH=CH2 [C=C]
CH2CH2Br [Br]
CH2CH2CN [CN]
COOH [COOH]
CHOHCH2OH [Diol]
OH OH OH OH
Si Si Si Si
O O O O
Derivatized
X X X X
O O O O
Si Si Si Si
O O O O
Si Si Si Si
O O O O
Glass [SiOH]
Close-packed Monolayer Surface

Fig. 1

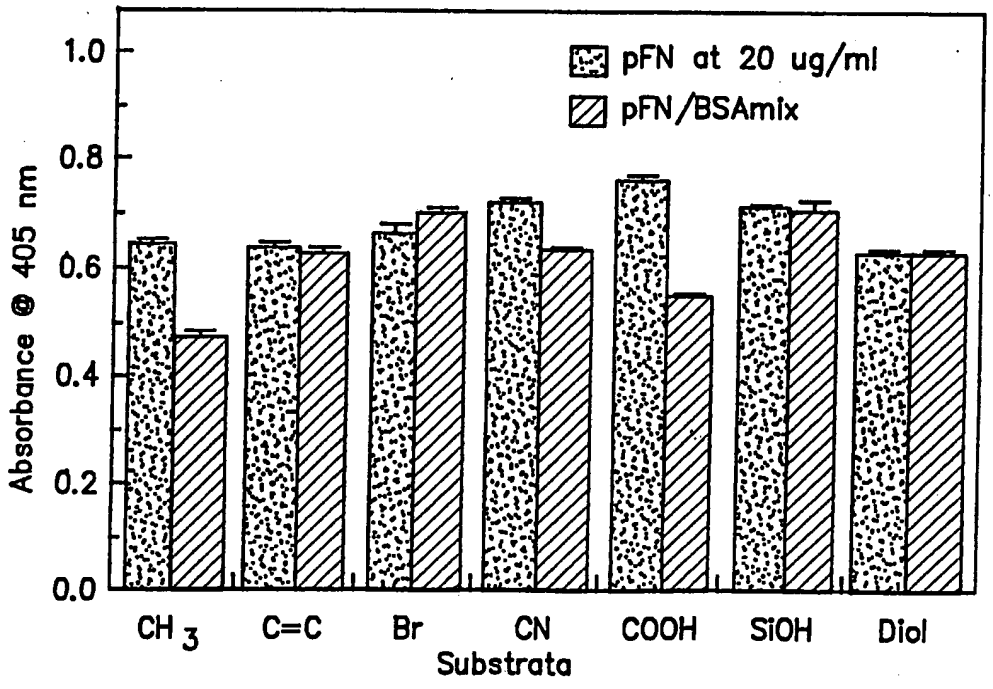
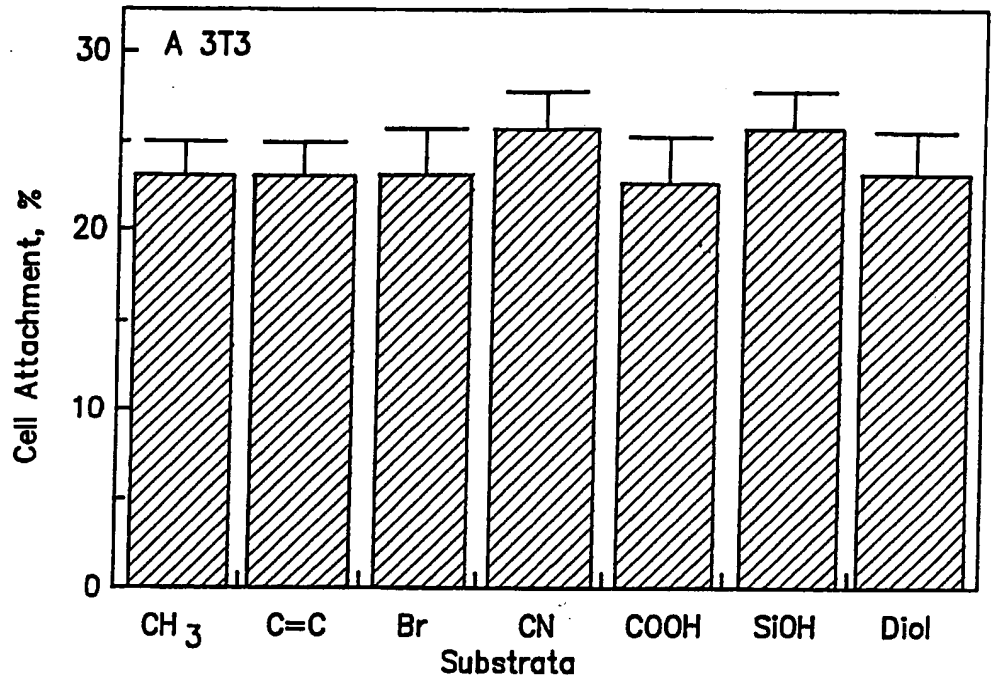

pFN at 20 ug/ml
pFN/BSAmix
Absorbance @ 405 nm
1.0
0.8
0.6
0.4
0.2
0.0
CH3
C=C
Br
CN
COOH
SiOH
Diol
Substrata

Fig. 2


A 3T3
Cell Attachment, %
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20
10
0
CH 3
C=C
Br
CN
COOH
SiOH
Diol
Substrata

Fig. 3A

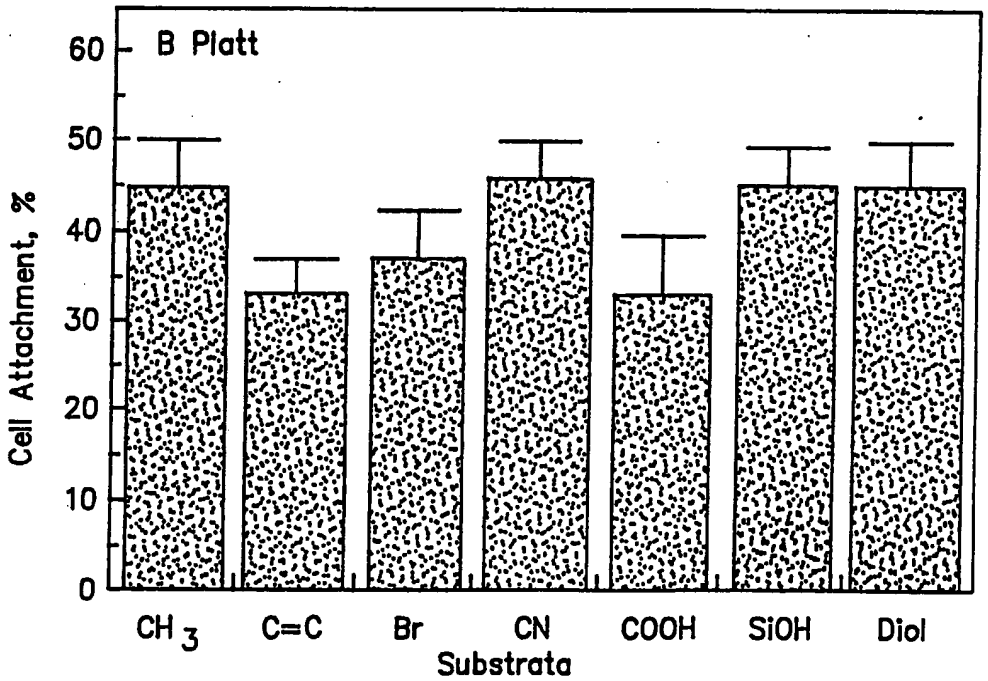
B Platt
Cell Attachment, %
60
50
40
30
20
10
0
CH 3
C=C
Br
CN
COOH
SiOH
Diol
Substrata

Fig. 3B

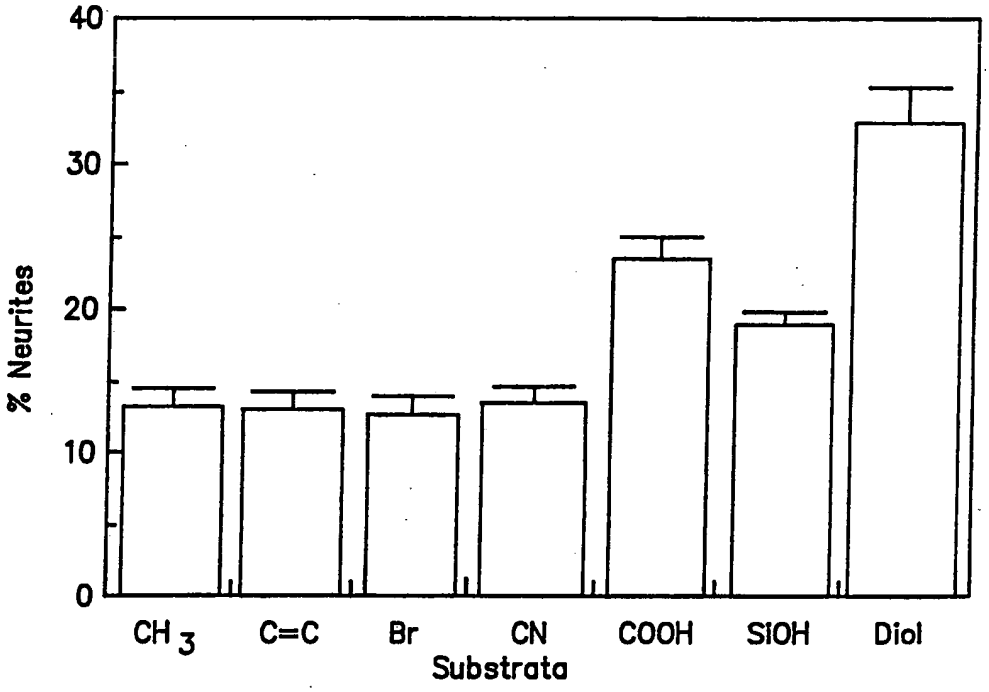

Fig. 4

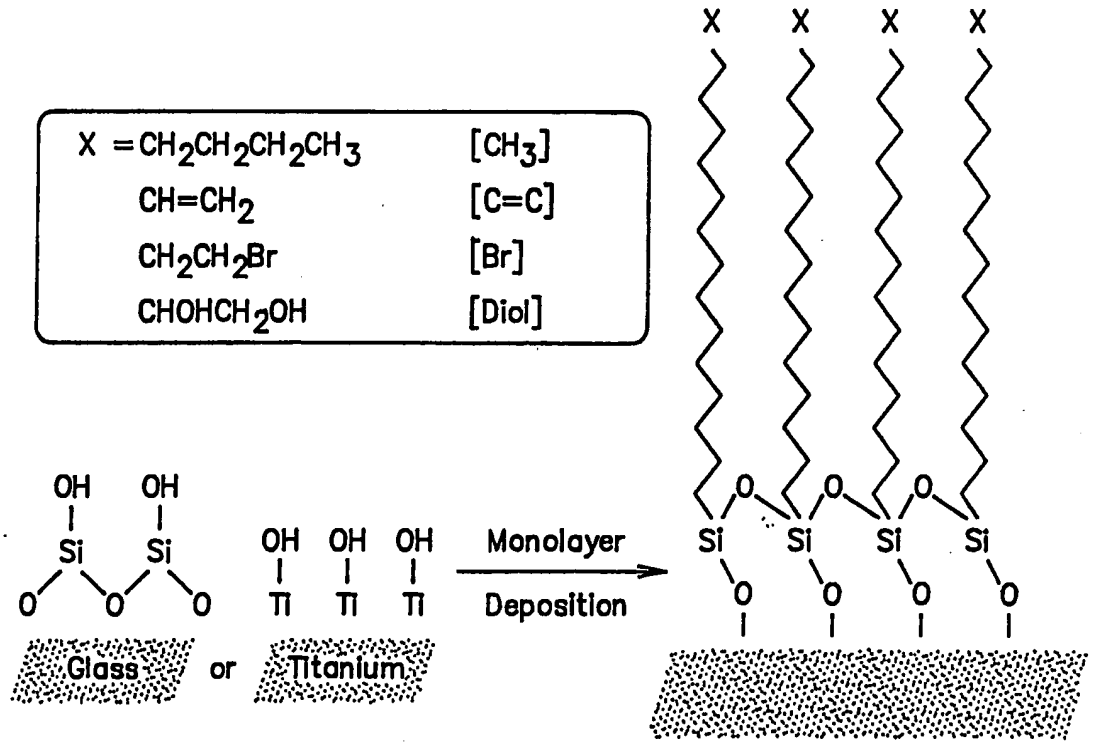

Fig. 5 Close-packed Monolayer Surface on Glass or Titanium

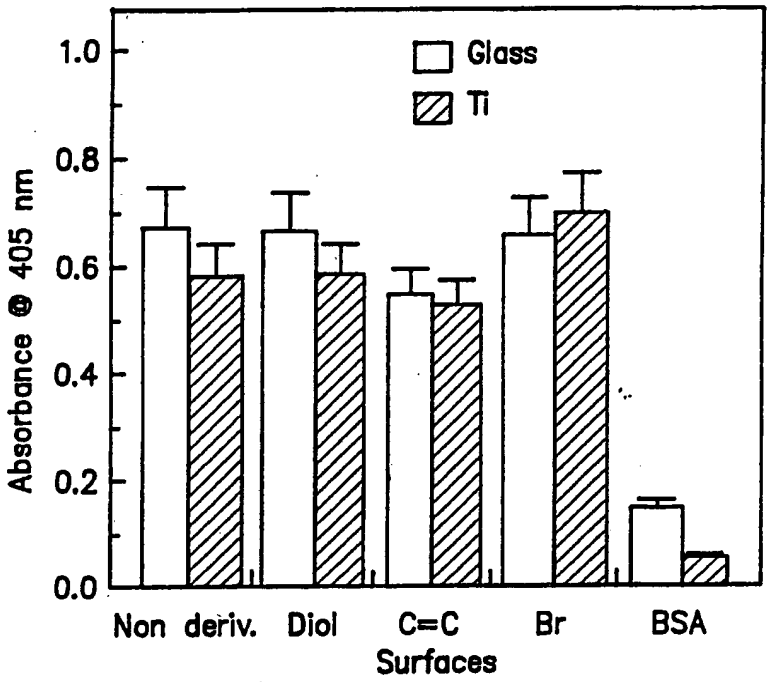
Glass
Ti
Absorbance @ 405 nm
1.0
0.8
0.6
0.4
0.2
0.0
Non deriv.
Diol
C=C
Br
BSA
Surfaces

Fig. 6

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/04466

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61F 2/ 54

U.S. CL.: 623/66

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S. CL.	623/11,66;427/2

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X Y	US,A, 4,878,563 (MUELLER-LIERHEIM) 09 MAY 1989; see the figure;column 1, lines 9-34;column 2, lines; 4-27; examples 1-6	1,3,4,10,14,16 17,19,30,32,33 36,38,39,42,44 45,40,50,AND 51 21-29
Y	US,A, 4,687,808 (JARRET et al.) 18 AUGUST 1987; see column 7, line 40 to column 8, line 21	21-29

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16 SEPTEMBER 1991	10 OCT 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	NGUYEN NGOC-HO For PAUL PREBILIC INTERNATIONAL DIVISION

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